Evaluation of the Applicator Dye Test for HTI Applicators

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Background

- Biomarkers of product usage are being used in microbicide studies to determine adherence to study products by study participants
- The dye applicator assay was developed by the Population Council for use with a Microlax applicator
- There are three published studies on the applicator assay which have reported high levels of sensitivity and specificity

Summary of Published Studies on the Applicator Assay

- Wallace et al. Pilot Study (Sex Transm Dis 2004; 31: 465) Reported that 40 applicators (20 used, 20 unused) could be correctly distinguished by 3 of 4 evaluators after staining with trypan blue
- Wallace et al. Feasibility Study (Contraception 2007; 76: 53) Evaluated FD&C Blue Dye on Microlax applicators filled with 2.5% methylcellulose in a clinically controlled setting
- Sensitivity 234/240 = 97.5% of inserted applicators stained blue
- Specificity 692/720 = 96% of non-inserted applicators did not stain blue

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Summary of Published Studies on the Applicator Assay

- Hogarty et al. Study (Sex Transm Dis 2007: 34) Evaluated 0.4% trypan blue staining of low density polyethylene HTI applicators containing 0.5% PRO2000/5 (P)
- Sensitivity 299/306 = 98-99% of inserted applicators staining blue
- Specificity 49/49 = 100% of non-inserted applicators did not stain blue

Applicator Assay Study Design

Positive Controls

- 70 single-use polypropylene HTI applicators
- 60 contained 0.1% UC781 or placebo gel, 10 empty

Negative Controls

- 70 single-use polypropylene HTI applicators
- 53 empty, 17 contained 2% SPL7013 (VivaGel)

Applicator Assay Study Design

- Prior to testing, one half of each patient-inserted applicator was swabbed with a cotton-tipped swab, moistened with sterile water and rolled onto a glass slide for Gram staining. Smears were assessed independently using oil-immersion microscopy for the presence of vaginal cells and bacteria to indicate the applicators had been inserted into the vagina.

- All applicators were stained with a 0.05% FD&C Blue Dye No. 1 solution (Prime Ingredients INC, Saddlebrook, NJ) and assessed independently to determine vaginal applicator use.

- The individual responsible for preparing and labeling the applicators did not serve as an evaluator.

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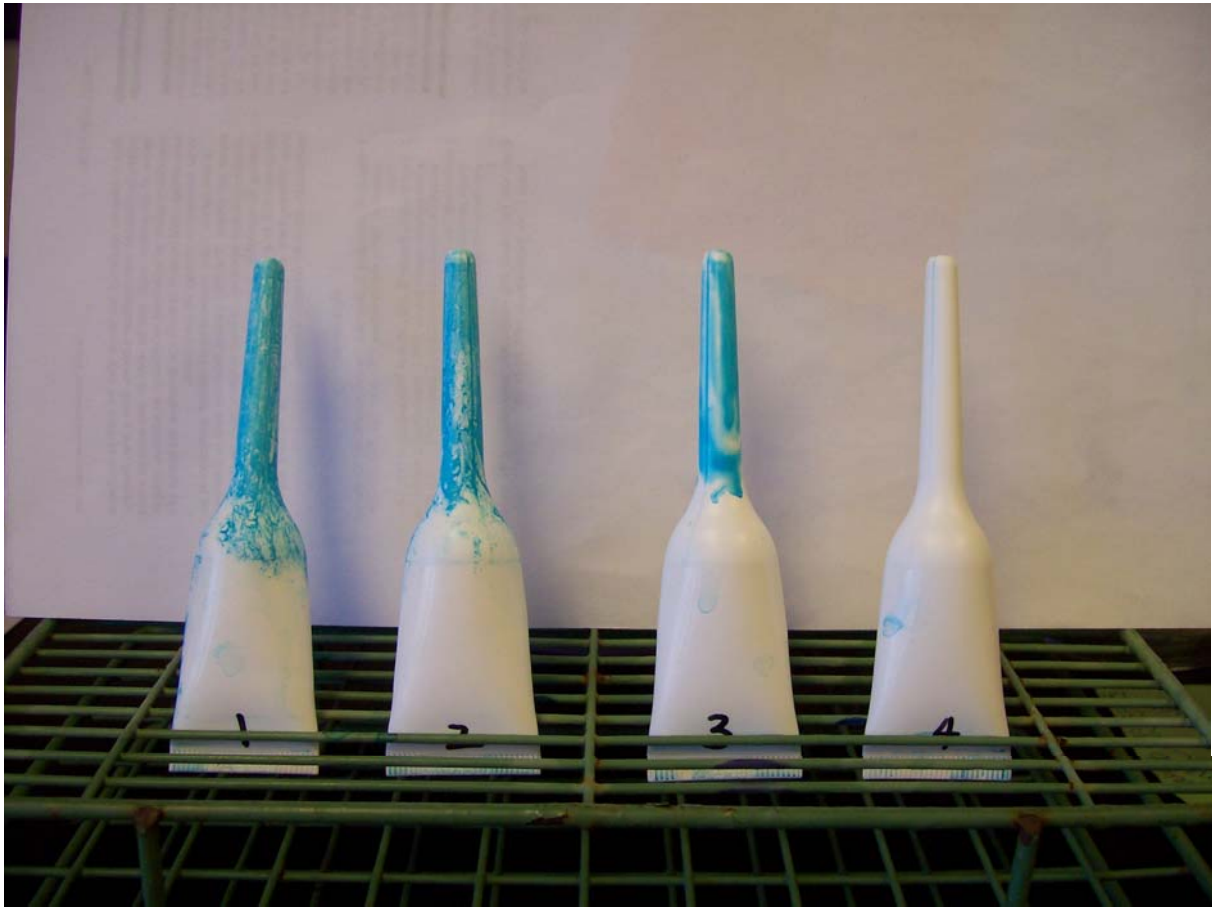
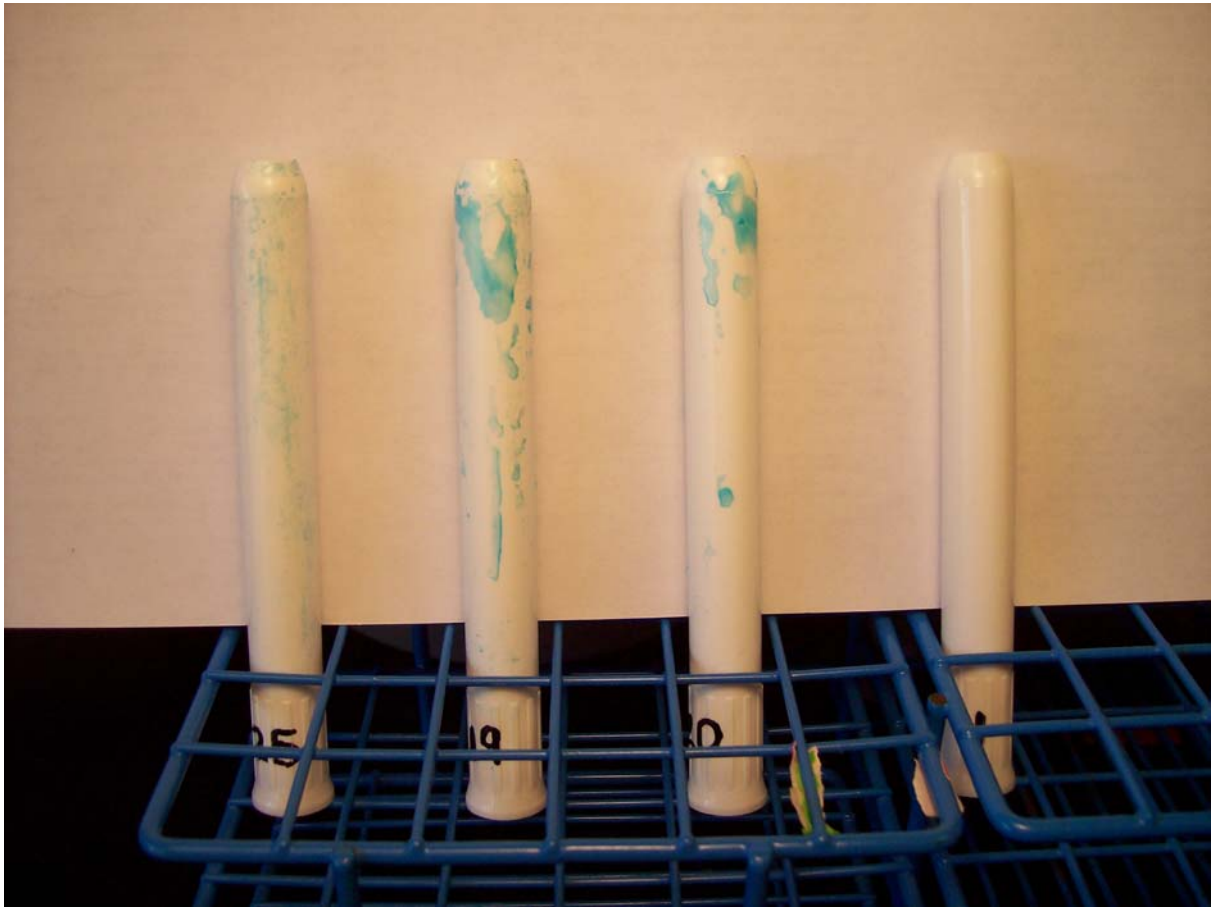
Microlax Applicators



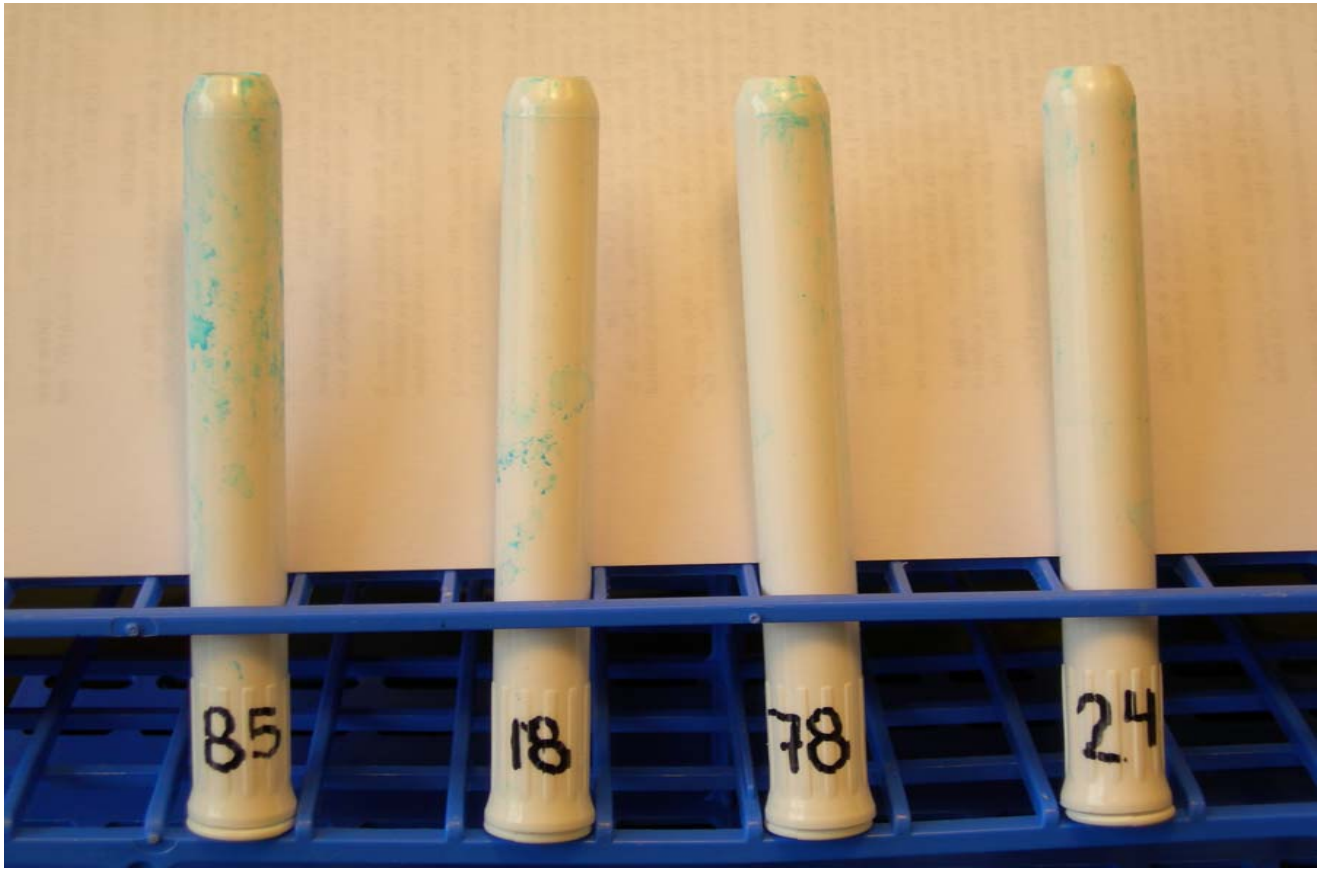
Used **Used/Gel** **Gel only** **Unused**

HTI Polypropylene Applicators



Used **Used/Gel** **Gel only** **Unused**

Applicators Containing UC781 Gel Inserted by Clinician



Clinician- Inserted Applicators

	Observer 1	Observer 2	Observer 3
Sensitivity	57/60 = 95%	56/60 = 93%	48/60 = 80%
Specificity	40/49 = 82%	47/49 = 96%	45/49 = 92%

MTN-004 Applicators

Applicator Assay Results: Patient-Inserted Applicators from MTN-004

	Observer 1	Observer 2	Observer 3
Sensitivity	130/169 = 77%	125/169 = 74%	80/169 = 47%
Specificity	17/17 = 100%	13/17 = 76%	16/17 = 94%

Applicator Assay Summary

- The dye-based applicator test has only 80-95% sensitivity and 82-96% specificity for detection of applicator insertion (clinician-inserted applicator) for the polypropylene HTI applicator
- The dye test performed even more poorly when women used gel daily; sensitivity ranged from 47-77% based on 3 independent observers

Acknowledgements

- The clinician-inserted applicators were provided through a clinical study of UC781 funded by NIAID/IPCP program
- The patient inserted swabs were generated by the first seven subjects enrolled in MTN-004.
- We thank the MTN Network Laboratory staff, Michele Austin, Ted Livant, and Hilary Shrader for their contributions